ORIGINAL RESEARCH PAPER

Dental Science

Volume - 7 | Issue - 1 | January - 2017 | ISSN - 2249-555X | IF : 3.919 | IC Value : 79.96

Comparative evaluation of probiotic ice-cream & normal ice-cream on salivary mutans streptococci count in children

KEYWORDS: Ice-cream, Probiotics, Mutans streptococci, Saliva

Dr Amee Alwani
Post graduate student, Department of Pedodontics and Preventive Dentistry -Corresponding author

Dr Karthik Venkataraghavan
Vibha dental care, 166, 2nd Cross, 2nd Stage, 2nd Cross Rd, Stage 2, Domlur, Bengaluru, Karnataka 560071

Dr Anup Panda
Head of the department, Department of Pedodontics and Preventive dentistry

Dr Krishna Trivedi
Senior lecturer, Department of Pedodontics and Preventive Dentistry

Dr Jina Jani
Post graduate student, Department of Pedodontics and Preventive Dentistry

Dr Jayati Dave
Post graduate student, Department of Pedodontics and Preventive Dentistry

ABSTRACT

Introduction: *Probiotics are live microorganisms which when administered in adequate amounts and confer a health benefit to the host. Dairy foods like cheese, curd, milk and ice-cream are considered useful vehicles for probiotic bacteria.*

Aim: *To compare the levels of salivary mutans streptococci before and after consumption of probiotic ice-cream.*

Materials and Methods: *20 school children who were caries free, in the age group of 10–12 years, were selected and divided into group I and II which were given 60 ml probiotic ice-cream and normal ice-cream for 7 days, respectively. Assessment of saliva samples was done at baseline, 1hr after consumption, and on the 7th day. Mitis Salivarius Bacitracin Agar was used for analysis. The number of colonies were counted and subjected to statistical analysis.*

Results: *The study showed mark reduction in salivary mutans streptococci counts in saliva after 1 h and on the 7th day in the probiotic group.*

Conclusion: *Pathogenic microorganisms could be displaced by probiotic bacteria. Thus, use of probiotic products could be exploited for the prevention of enamel demineralization.*

Introduction

Dental caries is one of the most prevalent diseases in humans, second only to the common cold.The economic burden for the treatment of this dental infection is staggering.[1-3] Although a disease of multifactorial origin, it is considered a result of the interplay of three principal factors: host, microflora and diet to which a fourth component time has been added. A group of phenotypically similar but genetically different streptococcal species, known as mutans streptococci, are considered the main etiological agents for dental caries in humans.[3-6] Based on DNA homology, mutans streptococci are divided into seven species: *Streptococcus mutans, S. sobrinus, S. ratti, S. riceti, S. downei, S. ferus, and S. macacae*; which can be subdivided into eight serotypes: a, b, c, d, e, f, g and h. Of these species, *S. mutans* and *S. sobrinus* have been implicated as the primary causative agents of dental caries in humans.[7]

Disease could be prevented not only by targeting the pathogen directly (e.g. with antimicrobial or antiadhesion agents) but also indirectly by interfering with the ecological pressure responsible for the selection of the pathogen.[8] Various methods were tried to suppress the caries activity. One of the novel strategies for prevention of dental caries is by manipulation of resident oral microorganism by ingestion of probiotic organisms.[9]

The interest in probiotic therapy to prevent oral diseases has grown remarkably over the years. PROBIOTIC, meaning 'For Life' was first coined in the 1960s, by Lilly & Stillwell.[10] Food and Agriculture Organization/World Health Organization ((FAO/WHO) in 2001 defined it as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host".[11] *Lactobacilli* and *Bifidobacterium* are the most commonly used and studied probiotic organisms. Replacement of cariogenic bacteria by non-pathogenic bacteria improved oral health in children, which can be done with the use of probiotics.

Dairy foods like cheese, yoghurt and milk are considered useful vehicles for probiotic bacteria, but an ideal administration vehicle has yet to be identified.[12] Ideally, it has been suggested that exposure early in life may facilitate a permanent installation of health promotion. Hence the probiotic vehicle should be suitable for all ages and especially for young children. In this context, ice-cream is an interesting probiotic food, as it is popular and universally liked.[13]

This study was carried out to compare the effect of consumption of probiotic containing ice-cream and normal ice-cream on levels of salivary mutans streptococci.

Materials and method

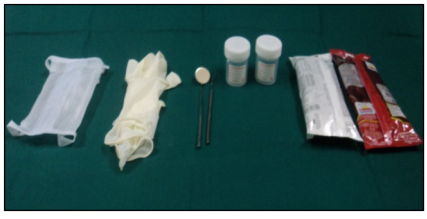


Figure:1 Materials used for study

Ethical clearance was obtained prior to the start of the study from the ethical committee. Informed consent from the parent/guardian was obtained. 20 school children with caries free teeth between ages 10-12 years selected for study.

Twenty children were randomly selected from the Gandhinagar

Volume - 7 | Issue - 1 | January - 2017 | ISSN - 2249-555X | IF : 3.919 | IC Value : 79.96

municipality school number-2 for the study. The children were equally divided by randomization into group I (probiotic ice-cream group) and group II (plain ice-cream group) comprising 10 children in each group, who were given 42 g (60 ml) probiotic ice-cream and plain ice-cream respectively for 7 days. Ice-cream was given once daily. The subjects were encouraged to maintain their normal oral hygiene habits. No tooth brushing was allowed for at least 1 h after eating the ice-cream.

Inclusion criteria

- Age group of the children 10–12 years
- No clinically detectable caries
- No history of any preventive dental treatment

Exclusion criteria

- Severely ill children
- Medically compromised children
- Children who had been on medication in the last 6 months
- Children undergoing orthodontic treatment

Collection of saliva was done after the clinical examination. Children were made comfortable and asked to swallow pre-existing saliva in order to clear the mouth of any residual saliva. Sterile hard plastic container was given to each student and the student was asked to split the saliva into it. The samples collected were handed over to the Biocare research laboratory for analysis on the same day. In the laboratory samples were stored at room temperature (17°C–25°C) prior to the analysis. Assessment of saliva was done at baseline, 1 hr and on the 7th day by using Mitis Salivarius Bacitracin Agar. The plates were incubated at 37°C anaerobically. Colony characteristics were studied after 72 h. Mutans Streptococci in saliva was determined by using a colony counter and the number of colony forming units was counted and result were analysed statistically.

Result

Excel and SPSS software packages were used for statistical analysis. The results were averaged (mean + standard deviation) for each parameter and parametric tests were used for statistical analysis.

In this study, mean salivary mutans streptococci count at baseline for probiotic ice-cream and plain ice-cream groups was 16048.80 and 16804.10 respectively. Mean salivary mutans streptococci count at 1 h after consumption of probiotic ice-cream and plain ice-cream was 12919.90 and 19742.80 respectively. When compared after 7 days, mean salivary mutans streptococci count after consumption of probiotic ice-cream and plain ice-cream was 9177.00 and 21043.70 respectively. When comparison of the mean salivary mutans streptococci was done 1 h and 7 days after consumption of probiotic and plain ice-cream the results were found to be statistically significant after 7 days. ($P \leq 0.05$)

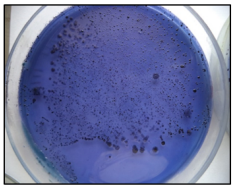
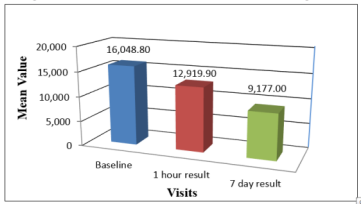
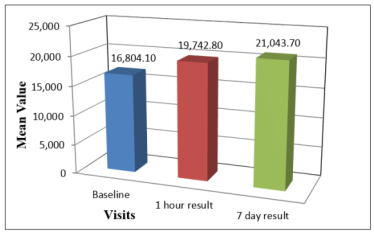


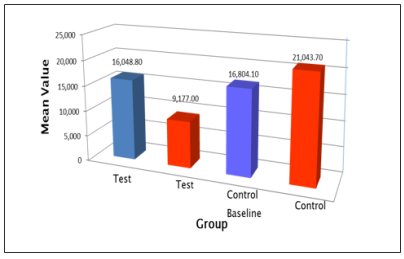
Figure : 2 MSB agar with mutans streptococci growth



Graph 1: Mean salivary mutans streptococci count for test group



Graph 2: Mean salivary mutans streptococci count for control group



Graph 3: Comparison of the number of colonies between test group and control group at baseline and on 7th day

	Group	N	Mean	Std. Deviation	P value
Baseline result	TEST	10	16,048.80	8,077.91	0.832
	CONTROL	10	16,804.10	7,595.87	
1 hour result	TEST	10	12,919.90	6,080.48	0.067
	CONTROL	10	19,742.80	9,269.34	
7 day result	TEST	10	9,177.00	5,873.86	0.002
	CONTROL	10	21,043.70	8,453.87	

Table 1 : Comparison of the number of colonies between test group and control group at baseline, at 1 hr and on 7th day

Discussion

Dental caries being a multifactorial disease process often requires a multifactorial approach to treatment and control. Bacteriotherapy is a novel and promising concept for combating infections and preventing dental caries.[14] Probiotic technology represents a breakthrough approach to maintain oral health by utilizing the natural beneficial bacteria commonly found in healthy mouth to provide natural defence against those bacteria which are thought to be harmful to teeth and gums.[15] To be effective against oral infections, probiotic bacteria need to adhere to the oral mucosa and dental tissues as part of the biofilm and compete with the growth of dental pathogens.[16] Studies have been performed to validate the survival and positive effects of *Bifidobacterium lactis Bb-12* within the human body, including immune response and gastrointestinal health in the young children.[17,18]

The effects of probiotics on the microbiota can be due to the competition for binding sites or secretion of antimicrobial components and lactic acid bacteria can produce different antimicrobial components.[19] Adhesion of probiotics in biofilms and co-aggregation with other bacteria has been demonstrated by in vitro studies.[20] Protection against pathogenic microbes and an enhanced maturation of the immune system can also be due to strengthening of the mucosal barrier, or effects on cytokine or chemokine production.[21,22] This in turn affects the cell-mediated and humoral immune responses, and partly explains the systemic effect produced by probiotics.

The administration of probiotics to small children may be difficult in the daily routines. Hence, different dairy products could serve as a vehicle for probiotics. Anti-cariogenic property is due to the presence of casein, calcium and phosphorus in the ice cream which adds the benefit to probiotic containing ice-cream and it is a dairy product that can be stored for a long time without any significant decrease in the number of viable probiotic cells.[23]

Volume - 7 | Issue - 1 | January - 2017 | ISSN - 2249-555X | IF : 3.919 | IC Value : 79.96

Ice-cream is a delicious, wholesome, nutritious frozen dairy product, which is widely consumed in different parts of the world by all age groups and especially children. Even though ice-cream has sweetening and flavouring agents, ice-cream has nutritional significance and could be an interesting carrier of probiotic food, with reported advantages.[24] Dairy products like milk and yogurt may not be liked by all the children and there are chances that children may not agree to continue with the study but ice-cream is universally accepted by children compared any other dairy products. Hence, Ice-cream was used as a vehicle in this study based on above mentioned reasons and it was well accepted by the participants.

On comparing the mean salivary mutans streptococci at baseline, after 1 h, and after 7 days of consumption of probiotic and normal ice-cream, the results were found to be statistically significant after 7 days.

Significant reduction in salivary mutans streptococci ($P \leq 0.05$) was found in our study after consumption of probiotic ice-cream, which was in accordance to the previous studies done by Chinnappa et al.[25], Caglar et al.[26], Cildir SK, et al.[27] and Zhu et al.[28] Singh et al.[13] also reported that probiotic ice-cream brought about a statistically significant reduction in mutans streptococci count. Similarly, Jindal et al.[29] concluded that statistically significant reduction in salivary mutans streptococci counts was recorded after probiotic ingestion. Whereas our results were in contrast to the previous study done by Chuang et al.[30] in which no differences in the counts of mutans streptococci between probiotic and control groups were found.

Comelli et al. examined 23 microorganisms used in the dairy industry for potential probiotic properties with respect to the prevention of dental caries. They showed that two S. thermophilus species and two L lactis species were able to adhere to hydroxyapatite. The authors concluded that such a property in a non-pathogenic dairy bacterial strain might prove beneficial in modulating the establishment of cariogenic dental plaque.[16]

During the period of test product consumption, continuously the mean value of mutans streptococci counts dropped, despite the sugar content of the ice cream. A caries-preventing effect of the probiotic organisms may have counteracted the caries-enhancing influence of the sugar, resulting in a reduction of the mutans streptococci count. The opposite was observed in the control group, which is probably because of the high sugar content and absence of probiotic organisms in the control product.[31]

In this study unstimulated saliva was collected from each participant, as the analysis of unstimulated saliva is more sensitive than analysis of stimulated saliva.[32] Moreover, stimulated saliva is less suitable for diagnostic applications because the foreign substances which are used to stimulate saliva tend to modulate salivary content and generally stimulate the water phase of saliva secretion, resulting in a dilution in the concentration of microbial count.[33]

Use of probiotics may reduce the cost of conventional therapy and prevention programs for the management of oral disease. It is an attractive idea of replacing harmful microorganisms with non-harmful, inactivated, or genetically modified bacteria.

Conclusion

Use of probiotic organisms for restoring oral health and prevention of caries is gaining interest. There is definitive effect of probiotic organisms in reducing the salivary levels of mutans streptococci for short time. Further studies on the long term or synergetic effect of the probiotic organisms on the caries causative bacteria, oral health and optimum dosage of the probiotic organisms are still need to be explored.

REFERENCES

1. Douglass, C. W., & Day, J. M. (1979). Cost and payment of dental services in the United States. Journal of dental education, 43(7), 330-348.
2. Gotowka, T. D. (1985). Economic growth of the dental profession: comparisons with other health care sectors. The Journal of the American Dental Association, 110(2), 179-187.
3. Loesche, W. J. (1986). Role of Streptococcus mutans in human dental decay. Microbiological reviews, 50(4), 353.
4. Loesche, W. J. (1985). Nutrition and dental decay in infants. The American journal of clinical nutrition, 41(2), 423-435.
5. Hamada, S., & Slade, H. D. (1980). Biology, immunology, and cariogenicity of Streptococcus mutans. Microbiological reviews, 44(2), 331.
6. Sánchez-Pérez, L., & Acosta-Gío, A. E. (2001). Caries risk assessment from dental plaque and salivary Streptococcus mutans counts on two culture media. Archives of oral biology, 46(1), 49-55.
7. Koga, T., Oho, T., Shimazaki, Y., & Nakano, Y. (2002). Immunization against dental caries. Vaccine, 20(16), 2027-2044.
8. Marsh, P. D. (2006). Dental plaque as a biofilm and a microbial community–implications for health and disease. BMC Oral health, 6(1), 1.
9. Meurman, J. H., & Stamatova, I. (2007). Probiotics: contributions to oral health. Oral diseases, 13(5), 443-451.
10. Lilly, D. M., & Stillwell, R. H. (1965). Probiotics: growth-promoting factors produced by microorganisms. Science, 147(3659), 747-748.
11. Hotel, A. C. P. (2001). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Prevention, 5(1).
12. Sameer, A. (2007). The synergism of probiotics in dentistry. Saudi Dent J, 17, 3.
13. Singh, R., Damle, S. G., & Chawla, A. (2011). Salivary mutans streptococci and lactobacilli modulations in young children on consumption of probiotic ice-cream containing Bifidobacterium lactis Bb12 and Lactobacillus acidophilus La5. Acta Odontologica Scandinavica, 69(6), 389-394.
14. Bhalla, M., Ingle, N. A., Kaur, N., & Yadav, P. (2015). Mutans streptococci estimation in saliva before and after consumption of probiotic curd among school children. Journal of International Society of Preventive and Community Dentistry, 5(1), 31.
15. Dhawan, R., & Dhawan, S. (2013). Role of probiotics on oral health: A randomized, double-blind, placebo-controlled study. Journal of Interdisciplinary Dentistry, 3(2), 71.
16. Comelli, E. M., Guggenheim, B., Stingele, F., & Neeser, J. R. (2002). Selection of dairy bacterial strains as probiotics for oral health. European journal of oral sciences, 110(3), 218-224.
17. Saavedra, J. M., Abi-Hanna, A., Moore, N., & Yolken, R. H. (2004). Long-term consumption of infant formulas containing live probiotic bacteria: tolerance and safety. The American journal of clinical nutrition, 79(2), 261-267.
18. Saavedra, J., Abi-Hanna, A., Moore, N., & Yolken, R. (1998). Effect of long term consumption of infant formulas with bifidobacteria (B) and S. thermophilus (ST) on stool patterns and diaper rash in infants. Journal of Pediatric Gastroenterology and Nutrition, 27(4), 483.
19. Silva, M., Jacobus, N. V., Deneke, C., & Gorbach, S. L. (1987). Antimicrobial substance from a human Lactobacillus strain. Antimicrobial agents and chemotherapy, 31(8), 1231-1233.
20. Stamatova, I., Kari, K., Vladimirov, S., & Meurman, J. H. (2009). In vitro evaluation of yoghurt starter lactobacilli and Lactobacillus rhamnosus GG adhesion to saliva - coated surfaces. Oral microbiology and immunology, 24(3), 218-223.
21. Caballero-Franco, C., Keller, K., De Simone, C., & Chadee, K. (2007). The VSL# 3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. American Journal of Physiology-Gastrointestinal and Liver Physiology, 292(1), G315-G322.
22. Latvala, S., Pietilä, T. E., Veckman, V., Kekkonen, R. A., Tynkkynen, S., Korpela, R., & Julkunen, I. (2008). Potentially probiotic bacteria induce efficient maturation but differential cytokine production in human monocyte-derived dendritic cells. World journal of gastroenterology: WJG, 14(36), 5570.
23. Bibby, B. G., Huang, C. T., Zero, D., Mundorff, S. A., & Little, M. F. (1980). Protective effect of milk against in vitro caries. Journal of dental research, 59(10), 1565-1570.
24. Hekmat, S., & McMAHON, D. J. (1992). Survival of Lactobacillus acidophilus and Bifidobacterium bifidum in ice cream for use as a probiotic food. Journal of dairy science, 75(6), 1415-1422.
25. Chinnappa, A., Konde, H., Konde, S., Raj, S., & Beena, J. P. (2013). Probiotics for future caries control: A short-term clinical study. Indian Journal of Dental Research, 24(5), 547.
26. Çaglar, E., Sandalli, N., Twetman, S., Kavaloglu, S., Ergeneli, S., & Selvi, S. (2005). Effect of yogurt with Bifidobacterium DN-173 010 on salivary mutans streptococci and lactobacilli in young adults. Acta Odontologica Scandinavica, 63(6), 317-320.
27. Cildir, S. K., Germec, D., Sandalli, N., Ozdemir, F. I., Arun, T., Twetman, S., & Caglar, E. (2009). Reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. The European Journal of Orthodontics, 31(4), 407-411.
28. Zhu, Y., Xiao, L., Shen, D., & Hao, Y. (2010). Competition between yogurt probiotics and periodontal pathogens in vitro. Acta Odontologica Scandinavica, 68(5), 261-268.
29. Jindal, G., Pandey, R. K., Agarwal, J., & Singh, M. (2011). A comparative evaluation of probiotics on salivary mutans streptococci counts in Indian children. European Archives of Paediatric Dentistry, 12(4), 211-215.
30. Chuang, L. C., Huang, C. S., Ou-Yang, L. W., & Lin, S. Y. (2011). Probiotic Lactobacillus paracasei effect on cariogenic bacterial flora. Clinical oral investigations, 15(4), 471-476.
31. Nagarajappa, R., Daryani, H., Sharda, A. J., Asawa, K., Batra, M., Sanadhya, S., & Ramesh, G. (2015). Effect of Chocobar Ice Cream Containing Bifidobacterium on Salivary Streptococcus mutans and Lactobacilli: A Randomised Controlled Trial. Oral Health & Preventive Dentistry, 13(3), 213-218.
32. Kaufman, E., & Lamster, I. B. (2002). The diagnostic applications of saliva—a review. Critical Reviews in Oral Biology & Medicine, 13(2), 197-212.
33. Miller, C. S., Foley, J. D., Bailey, A. L., Campell, C. L., Humphries, R. L., Christodoulides, N., ... & Redding, S. W. (2010). Current developments in salivary diagnostics. Biomarkers in medicine, 4(1), 171-189.